processing signals are required for the antigen-presenting cell to properly process the antigen and display it on its surface. Such processing is accomplished by the cellular endosomes. Proper signals on both the N-terminal and C-terminal portion of the protein are believed to be required. These can be supplied, *inter alias*, using the signal and transmembrane and cytoplasmic tail portions from LAMP-1 and LAMP-2 genes. Such signals are known in the art.--

REMARKS

Claims 41-67 are presently pending. The specification has been amended to correct an obvious typographical error. The extracellular domain of the α subunit of AChR extends from amino acid 1 to amino acid 210 (not 120). The fact that the extracellular domain extends to amino acid 210 is repeated numerous times in the specification. See, for example, page 11, line 9 and again on line 22, and page 17, line 5. No new matter is introduced by the amendment to the specification.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made."

Rejection of Claims Under 35 USC § 112, First Paragraph

Claims 41-67 are rejected under §112, first paragraph as not enabled. The Office Action reiterates that Orkin *et al.* addresses *ex vivo* therapy as a form of gene therapy, (page 3), and that Crystal *et al.* "stated that the choice of an ex vivo or in vivo strategy and of the vector is dictated by the clinical target. (See page 404, paragraph 3)." The Office Action further asserts that there is unpredictability in the art because Crystal

> stated that there is significant variation that exists in the genetically marked cells recovered from recipients in *ex vivo* studies, emphasizing the unpredictability in the art. (See page 405, col. 3, paragraph 3). Crystal explicitly states that results even in *ex vivo* methods are inconsistent. (See page 409, col. 1 lines 41-43).

Page 3. The Office Action indicates that a particularly high level of enabling description is therefore required in this application.

Applicant respectfully traverses. The fact that Orkin might have discussed *ex vivo* treatments in general or that a clinical target appears to be more favorably approached *ex vivo* or *in vivo* or by some particularly well adapted vector does not appear particularly germane to the facts of the present invention. Those considerations regarding the state of the *ex vivo* art generally do not relate to the specific facts of the present invention. The invention is based on *ex vivo* gene transfer. Any nucleic acid vector can be introduced into cultured cells. The claims recite transferring *in vitro* the gene(s) of interest into the patient's own antigen presenting cells (APCs) and reintroducing those APCs into the patient. Therefore concerns regarding *in vivo* gene transfer such as, for example, targeting of proper cells, are not germane. The *in vitro* transfer of the gene permits isolation of cells which are transfected, and, if need be, assessment of the expression levels before treatment of the patient with the cells. The fact that a patient's own cells are introduced eliminates possible undesirable immune responses to the engineered cells.

The remaining statements of the Office Action regarding Crystal's teachings are misleading. Read in context, the cited statements of Crystal emphasize that positive *ex vivo* results were obtained. For example, the relevant paragraph at page 405, col. 3, paragraph 3, states:

> Although there is variation among ex vivo clinical trials in the proportion of genetically marked cells recovered from the recipients, retroviral vector DNA or marker gene-derived mRNA or both have been observed in cells collected after periods ranging from several weeks to 36 months after administration.

Therefore, *ex vivo* clinical trials have demonstrated the presence of a treatment agent for significant periods of time. Similarly, the quote at page 409 states: " In most of the ex vivo marrow-marking trials, successful gene transfer is observed intermittently (Table 1)." Table 1 is titled "Summary of studies showing that transfer of genes to humans is feasible." The results in the table specific to marrow show that there was evidence *in vivo* of gene transfer for at least 5 months to as long as 36 months. Therefore, Crystal's remarks weigh in favor of the successful practice and therefore the predictability in the art.

Specifically in regard to the invention, the Office Action asserts that:

> being in a new field, the state of the prior art does not teach one skilled in the art how to transfer a gene and induce expression at a level sufficient to achieve a therapeutic response with each and every vector. Applicants directed examiner to page 7 of the specification, which, contrary to the applicant's argument, specifically state applicant's prophetic statements of the ability of any viral vector to transfer the gene of interest. This confirms that applicants are not enabled for each and every viral vector.

Page 3.

Applicant respectfully traverses. The use of prophetic statements is perfectly permissible and does not establish or confirm a lack of enablement. No such inference is proper. Enablement does not require actual reduction to practice nor exemplification of each and every viral vector.

In the present invention a gene encoding an antigen to be presented to a specific T-cell population is introduced into antigen presenting cells (APCs) in culture. The APCs transfected or transduced with the gene of interest are transferred back to the patient. See, *inter alia*, page 7, lines 5-6, and page 8, lines 17-20. The expression of the gene can be monitored and assessed in culture. See, *inter alia*, page 14, lines 7-12. The applicant has given an example and experimental results of how this works with plasmid vectors. Pages 9-15. In a recent article by the applicant, excellent results with the plasmid-based experimental system are described:

> Our results show for the first time that APCs can be engineered to process and present an autoantigen, and will effectively target the spectrum of T cells related to the autoimmune disease. In these experiments we used the domain of TAChR to which the majority of T cells respond, i.e. the extracellular 1-210 amino acid sequence of the α-subunit, as the antigen. T cell proliferative responses were obtained with APCs presenting either endogenous or exogenous antigen. However, the endogenous pathway was the more efficient one in terms of eliciting T cell proliferative responses. It is striking that as few as 200-1000 transfected A20 cells maximally stimulated 2.5×10^4 T cells.

Wu *et al., J. Neuro.* 106: 145-153 (2000), at page 151. As reported, the efficacy of the system was striking with each APC resulting in maximum stimulation of from 25 to over 100 T cells ($2.5 \times 10^4 \div 200$ to 1,000).

Vaccinia virus vectors ("VVV") were another example of vector taught in the specification. VVV have long been used as a vector for expression of genes in mammalian cells including APCs. Macket *et al., Proc. Natn'l Acad. Sci.* 79: 7415-9 (1990) and Wu *et al., Proc. Natn'l Acad. Sci.* 92: 11671-5 (1995). The Office Action fails to present a prima facie showing that a viral vector would not work as well as a plasmid vector.

The attached Declaration under 37 CFR §132 provides experimental data which demonstrate that VVV can be engineered and used in culture to transduce APCs effectively. The VVV induce a high level of expression of an antigen, of FasL, and of truncated FADD, sufficient to enable complete targeting and specific killing of relevant T-cells and protection of APCs. Sufficient levels of expression are achieved from a variety of constructs, as demonstrated by the growth inhibition and apoptosis of specifically targeted T cells. Two different antigens were targeted to T cells in the tests presented in the attached Declaration. Applicant believes that the results dispel any doubts as to the ability to use virus vectors such as VVV for in-culture transfection, and respectfully requests indication to this effect.

The Office Action further asserts that the specification does not teach how to use the plasmid vector or the VVV for therapy, or how to use the cultured cells. Page 4.

Applicant respectfully traverses. *Ex vivo* treatments generally are well known in the art. Cell culture methods are also known and routine. Cell transfection and transduction methods are well known and routine. For example, prior to filing of the present application, long term *in vivo* gene expression had been obtained and reported for marker genes from *ex vivo* transformed hematopoietic progenitor cells. See Abstract, Brenner *et al., Cyto. Mol. Ther.* 2: 193-200 (1996). In addition, Dabeva *et al., Proc.*

Natn'l. Acad. Sci. 94:7356 (July, 1997)) demonstrate the state of the art of *ex vivo* treatments. They report collection of human pancreatic cells, selection of a desirable subpopulation, growing the cells in culture, and successful differentiation and function *in vivo* when reimplanted in liver. See Abstract. An article published in 1999 indicates the extent of use of *ex vivo* procedures at the time of the invention. It describes a review of 203 patients undergoing autologous peripheral blood progenitor cell transplants in the year prior to filing this patent application. Boldwell *et al., Bone Marrow Transplantation* 24: 53-55 (1999). Each of those articles relate to *ex vivo* uses. They demonstrate the extensive and common use of the basic techniques, cell isolation, cell transduction or transformations, cell culture, and re-implantation, in use at the time of filling of the present application.

Lack of *in vivo* data is not in itself a bar to patenting. The cultured cell data presented in the specification and the declaration demonstrate the successful use of both plasmid and viral vectors to create a level of expression sufficient to induce T cell apoptosis. The claimed *ex vivo* methods avoid obstacles typical for *in vivo* therapy, such as immune rejection of the vector or lack of targeting of a cell by the transfecting polynucleotide.

The Office Action asserts that use of gene fragments (the extracellular domain of the α subunit of AChR) is not enabled given only the prophetic statement by applicant that they express epitopes to which most AChR-specific T-cells respond. Page 4.

Applicant respectfully traverses. The specification specifically teaches the use of genetic constructs in which the extracellular domain is expressed and successfully presented in culture to T-cells specific for the total AChR protein. The Declaration accompanying this response demonstrates that T-cells were maximally stimulated and virtually all killed when genetic constructs expressed amino acids 1-210 of AChR. The Office Action does not provide any reasons to lead one to doubt that the effect on T cells *in vivo* would be similar. Thus the PTO has not met its burden of presenting a *prima facie* showing. There is no reason to expect that a protein domain shown to be sufficient

when expressed on cells in culture would not be sufficient when expressed on cells *in vivo*.

Applicant respectfully requests withdrawing the rejection of claims under 35 USC § 112 as the PTO has failed to meet its burden by providing prima facie evidence to doubt applicants presumptively accurate assertions.

In light of the comments presented above, it is respectfully urged that the present claims are in condition for allowance. Notice to that effect is earnestly solicited.

Respectfully submitted,

Date: January 26, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 6, line 16, has been amended as follows:

All or a part of the coding sequence for acetylcholine receptor is transduced or transfected into the APCs. This can be accomplished by any technique known in the art, including using viral vectors or plasmid vectors. The extracellular portion of the α-subunit (comprising amino acids 1-210 ~~1-120~~) is believed to comprise the epitopes to which most AChR-specific T cells respond. Thus this portion is believed to be sufficient as the presented antigen for the antigen presenting cells. Moreover, it is believed that proper processing signals are required for the antigen-presenting cell to properly process the antigen and display it on its surface. Such processing is accomplished by the cellular endosomes. Proper signals on both the N-terminal and C-terminal portion of the protein are believed to be required. These can be supplied, *inter alias*, using the signal and transmembrane and cytoplasmic tail portions from LAMP-1 and LAMP-2 genes. Such signals are known in the art.